

Biotooth – A Dream or Reality

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Abstract

Loss of a tooth has a huge psychological effects as it adversely affects esthetics of one's face along with the mouth functions. Replacement of missing or damaged teeth usually involves fixed or removable prostheses or dental implants. The use of dental implants is the most rapidly growing area of dentistry. But these current treatment methods can, sometimes, reduce the quality of life because of their limited physiological function, or elicit an immunological rejection. Implants are more difficult and less successful when tooth loss is accompanied by bone loss. Here, this article outlines the current approaches toward the tooth regeneration, and focuses on several key challenges that must be met in the making of a bio-tooth.

Key Words: *Implants, Stem cells, Biotooth, Tissue engineering*

Introduction

A biological tooth (bio-tooth) that is made from the patient's own cells and grows in its intended location should be the best choice for treating the tooth loss. A biotooth is a genetically engineered tooth created from stem cells. The stem cells for a biotooth are obtained from primary tooth, unerupted tooth bud, third molar, chord cells (blood) and tissue engineered cells (adipose tissue, hair follicle). These are the cells that regenerate via mitotic cell division and differentiate into specialized cell types. Stem cells have the remarkable potential to develop into many different cell types in the body during early life and growth on simulation.

These cells have unique properties of:

1. **Self-renewal:** Stem cells can renew themselves almost indefinitely which is also known as proliferation.
2. **Differentiation:** Stem cells have the special ability to differentiate into cells with specialized characteristics and function.
3. **Unspecialized:** Stem cells are largely unspecialized cells which then give rise to specialized cells.

Types of Stem Cells

Based on their origin, stem cells are categorized either as

1. Embryonic stem cells (ESCs) or as postnatal stem cells.
2. Somatic stem cells or adult stem cells.

Embryonic stem cells are best derived from 2-11 days old embryo called blastocyst. ESCs are considered as immortal as they can be propagated and maintained in an undifferentiated state indefinitely. The therapeutic benefit of these totipotent cells is curtailed by moral and ethical concerns as extracting stem cells from an embryo destroys the embryo itself. Owing to these shortfalls, ESCs remained only as platform for research¹.

Adult stem cells are multipotent, i.e., they are capable of differentiating into more than one cell type but not all cell types. Moreover they have plasticity, that is, their ability to expand beyond their recognized potential irrespective of the parent cell from which they are derived. Adult stem cells can be hemopoietic stem cells (HSCs) or mesenchymal stem cells (MSCs)².

For Tissue engineering, following three factors play a major role:

1. **Morphogenic Signals** such as growth factors and differentiation factors which play an important role in the multiplication

and differentiation of stem cells into the specifically needed type of cells. BMPs (bone morphogenic proteins) and cytokines play a major role in organogenesis, e.g. in differentiation of dental pulp stem cells into odontoblasts which is the main requirement of teeth tissue engineering³.

2. **Responding stem cells** which are originally obtained from the patient and preserved under good conditions to maintain their special ability to differentiate into a wide range of cells.
3. **Scaffold (Fig. 1)** provides physical support to the cells required for regeneration of any tissue. It provides these cells with the environment to grow. These scaffolds should be biodegradable and rate of degradation should coincide with the rate of tissue formation. They should be porous and allow appropriate differentiation of cells without affecting their progeny.



Fig. 1: Scaffold in bio-tooth formation. (Source: Columbia University Medical Center)

Transformation from Xenodontics - Biodontics

1. **Biomembrane scaffolds** are seeded with stem cells implanted in the jaw at socket or prepared site. (BMSC and DPSC) scaffold may be collagen hydrogel, chitosan, poly-L-Lactic acid, poly-L-Glycolic acid, HA+TCP.
2. **Scaffold implantation** done (Orthotopic or ectopic) by soak system, low pressure system, pipette system or syringe system. Osteogenic differentiation takes around 2 weeks

3. **Osteogenic differentiation**-SDF1 and BMP 7 plays role in angiogenesis



4. **Positional information and tooth morphogenesis** (barx1, 3-D bioprinting, EDA, TRAF6 play role.



5. Bone regeneration and periodontal ligament regeneration

Bio Tooth replacement

This procedure is a better alternative than traditional teeth replacement methods and it involves regrowing or reconstruction of a tooth in the mouth. However, the problem with this procedure is that it needs the right tooth shape. Following are the ways in constructing a bio tooth:

1. Reconstruct the mature tooth as it appears in the mouth.
2. Reproduce the embryonic development in the mouth.
3. Induce a third dentition.
4. Create a tooth shaped scaffold, place some cells in them and wait for the cells to grow.

Of the options present above, only two are being tried in the conceptual stage and the other two are still being studied.

Reconstruct the mature tooth as it appears in the mouth: The four components in a tooth- the crown, dental pulp, enamel and root- are separately constructed from the materials and right cells.

The drawback of this procedure is that the process has a high level of technical difficulty. The advantages, on the other hand, include having a high level of control on the process and the possible automation and scale-up.

Inducing a Third dentition: This prospect has been around for quite a while, and there is an appealing proposition for this. Primarily, it works by adding molecules with either of the two earlier dentitions in development of initiating the de novo of the tooth post tooth loss. But there are two problems encountered: genes involved in induction of tooth initiation have a part in bone development, and other cellular process and cells found in the teeth are not present in the adult jaw, therefore the molecules have nothing to act upon.

Create a tooth shaped scaffold, place some cells in them, and let the cells grow: This procedure is highly successful, and uses tissue engineering technique. It involves seeding of biodegradable scaffolding with cells, and generation of these tissues will mold on to the shape of the scaffolding. There are various uses of these scaffolds, and may even be able to regenerate teeth and other organs, but this theory is still under investigation.

Applications in dentistry: In the field of dentistry, stem cell research is directed towards achieving the following; regeneration of damaged coronal dentine, pulp, resorbed roots, cervical or apical dentine and periodontal ligament; besides plugging of perforations, repair of craniofacial defects and whole tooth regeneration.

Dental pulp stem cells (DPSCs) (Fig. 2) represent a kind of adult cell colony which has the potent capacity of self-renewal and multiline differentiation (Fig. 3). These stem cells seem to be the source of odontoblasts that contribute to the formation of dentin pulp complex. Some studies have proved that DPSCs are capable of producing dental tissues in vivo including dentin, pulp and crown like structures, where as other investigations suggested that these stem cells can bring about formation of bonelike structures. Theoretically, a bio-tooth made from autogenous PSCs should be the best choice for clinical tooth reconstruction⁴.

Granthos et al⁵ demonstrated, both in vitro and in vivo, in animals that dental pulp stem cells (DPSCs) were capable of forming ectopic dentin and associated pulp tissue.

Batouli et al⁶ used an in vivo stem cell transplantation system to investigate differential regulation mechanisms of bone marrow stromal stem cells (BMSCs) and DPSCs. DPSCs were found to be able to generate a reparative dentine like tissue on the surface of human dentin in vivo.

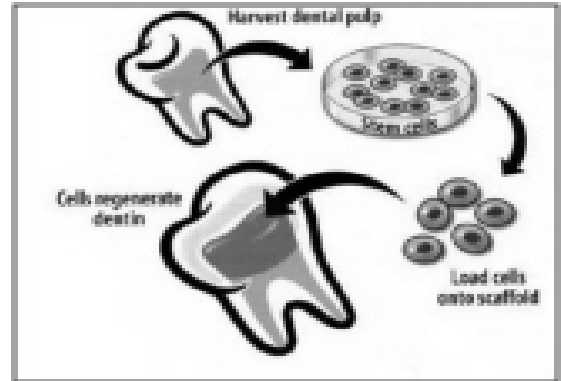


Fig. 2: Dental pulp stem cells (DPSCs) and regeneration of damaged coronal dentine and pulp (Source: Krebsbach PH, Robey PG. Dental and skeletal stem cells: potential cellular therapeutics for craniofacial regeneration. J Dent Educ. 2002 Jun;66(6):766-73)

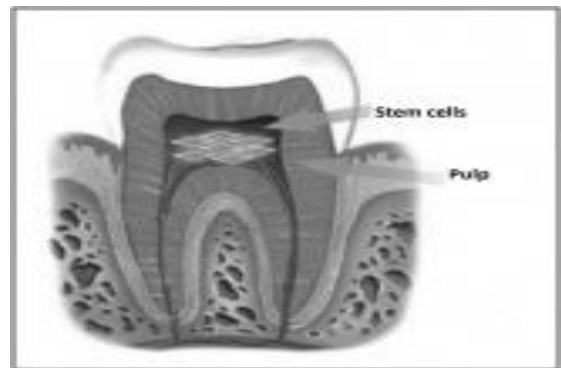


Fig. 3: Cells will be able to regenerate in future from pulp. (Stem saves: National Institute Of Health: The human body)

Regenerating periodontal ligament: Periodontal regeneration has always remained a challenge as it consists of cells that give rise to both hard and soft tissues. Kawaguchi et al (2004) used autologous bone marrow (MSC) in combination with allocollagen to regenerate periodontal ligament in experimental grade III defects in dogs. One month after implantation, there was regeneration of cementum, periodontal ligament, and alveolar bone⁷. Hasegawa et al⁸ demonstrated that autologous periodontal ligament cells cultured in vitro were successfully reimplanted into periodontal defects in order to promote periodontal regeneration in dogs and a subsequent study confirmed this evidence in humans.

Bio- Tooth Generation: Replacing a missing tooth has always been a challenge in the field of dentistry. Teeth have also been engineered

ectopically and transplanted into the jaw with some success⁹. Recently some researchers developed a bio root into which a post and crown were placed. This further developed a natural relationship with bone. Complete tooth regeneration can also be accomplished by placing the stem cells into a mold of tooth crown which is made of enamel-like substance with a scaffold material. The stem cells then start looping blood vessels through this scaffold to enable its implantation elsewhere in the body until mature teeth are formed, following which these teeth will be extracted and implanted in the oral cavity¹⁰.

Craniofacial Defects: Craniofacial bone grafting procedures rely on autologous bone grafting, devitalized allogenic bone grafting and natural/synthetic osseous conductive biomaterials. Stem cell transplantation procedures have been studied to replace these procedures in future¹¹. Adult stem cells can be harvested from the bone marrow and expanded in the laboratory.

When loaded onto appropriate scaffolds and transplanted back into a deficient site, stem cells have the potential to regenerate bone structures.

Dental stem cell banking^{12,13}: With the advancement of cryopreservation technology, the first commercial tooth bank was established in 2004 at National Hiroshima University, Japan. Presently, Stem Cell Banks freeze not only cord stem cells but also dental stem cells of baby teeth. This can be done easily when a child's anterior milk tooth is shedding. After the extraction of the tooth, it is preserved in a special kit provided from the stem cell bank company which transfers the tooth to their special labs to harvest the dental stem cells and store them in their bank for each child, confidentially, until they are needed later for the child himself or a member of his family.

Conclusion

Although such bioengineered teeth have been nothing more than a dream for many centuries. In the near future dental stem cells will probably be able to grow new teeth and jaw bone, thereby enabling unlimited therapeutic applications. However issues involving in the reconstruction of a bio-tooth regarding the shape determination, size control, availability of dental epithelium, directional growth and eruption, and graft rejection in the jaws remain to be resolved. Various challenges must be overcome before this

novel therapy can be translated from labs to clinics. Collaboration between basic scientists and clinicians is required to achieve this goal. Advancement in the field of research have revealed a ray of hope to dentistry for the generation of a biotooth in humans that will improve quality of life in the upcoming years.

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